

August 19, 1997

VETERINARY SERVICES MEMORANDUM NO. 800.73

Subject: General Requirements For Immunodiagnostic Test Kits

To: Veterinary Biologics Licensees, Permittees, and Applicants
Director, Licensing and Policy Development, Center for Veterinary Biologics
Director, Laboratory, Center for Veterinary Biologics
Director, Inspection and Compliance, Center for Veterinary Biologics

I. PURPOSE

The purpose of this memorandum is to provide guidance for licensing immunodiagnostic test kits, and to specify requirements for the components commonly included in such kits.

II. CANCELLATION

Veterinary Services Memorandum No. 800.73, dated January 7, 1986, is hereby rescinded.

III. GENERAL REQUIREMENTS

When prescribed in an applicable Standard Requirement or filed Outline of Production, Immunodiagnostic test kits shall be characterized in accordance with the requirements prescribed in this section.

A. Outline of Production - A description of the preparation of kit components and reagents shall be as specified in Title 9, Code of Federal Regulations (9 CFR), Part 114.9(f), Outline Guide for Diagnostic Test Kits.

B. Master Seeds and Ingredients:

1. Each lot of Master Seed virus shall be tested for bacteria and fungi as specified in 9 CFR 113.27, and extraneous viruses as prescribed in 9 CFR 113.55. Each lot of Master Seed bacteria shall be tested for bacteria and fungi as specified in 9 CFR, 113.27(b) and appropriate biochemical and cultural characteristics as specified in the filed Outline of Production.

2. Extracted and synthetic antigens used as Master Seed shall be characterized by appropriate laboratory procedures and described in protocols acceptable to and filed with APHIS.

3. Ingredients of animal origin and reagents obtained from foreign sources shall be subject to the requirements and restrictions specified in 9 CFR Parts 104 and 122; and 9 CFR 113.50 and 113.53.

4. Cell Culture Requirements - Cell cultures used in Master Seed preparation shall meet the applicable requirements prescribed in 9 CFR 113.51 for primary cells; 9 CFR 113.52 for cell lines, and 113.53 for ingredients of animal origin.

IV. COMPONENTS OF IMMUNODIAGNOSTIC TEST KITS

Components of test kits are subject to the requirements, and restrictions outlined in the following chart:

Components	Manufactured in Licensed Premises	Same Lot and Lot Controlled for Entire Serial	Source of Material and/or Formula in Outline	Data Submitted Before Changing Source/Formula	Dating of Serial
Anti-species Antibody or Conjugate ^A	No	Yes	Yes ^{D,E}	Yes ^{D,E}	Yes
Agent Antigen or Antibody ^B	Yes ^C	Yes	Yes ^{D,E}	Yes ^{D,E}	Yes
Sample Diluent	No	No	Yes ^{D,E}	Yes ^{D,E}	No
Control Sera (or Antigen)	No	Yes	Yes ^{D,E}	Yes ^{D,E}	Yes
Plates	No	Yes	Yes ^{D,E}	Yes ^{D,E}	No
Stop Solution	No	No	Yes ^{D,E}	Yes ^{D,E}	No
Coated Plates	Yes	Yes	N/A	N/A	Yes

A. Anti-species antibody, protein A, biotin, or a labeled version of any of the above, etc. Non-antigen-specific reagent to coat plate or amplify/report immune reaction (e.g., "conjugate").

B. Agent antigen or anti-agent antibody or conjugate to coat plates or to compete with or participate in the specific agent antigen-antibody reaction.

C. If produced from an APHIS qualified master seed or master cell stock in a licensed facility, then no additional testing is required. If produced at other than a licensed facility, then each lot of antigen or antibody requires appropriate validation. Sufficient quantities should be available to allow confirmatory testing at the CVB-L.

D. The Outline of Production must be amended prior to receiving approval to use alternative formulations and prior to receiving approval to obtain the indicated component from an alternative source.

E. Provide sensitivity, specificity and suitability data as prescribed by APHIS.

V. PRELICENSING REQUIREMENTS

The primary licensing considerations (performance criteria) for diagnostic test kits are:

A. Sensitivity - Expressed as the percentage of positive results from specimens obtained from animals known to have the disease. Sensitivity is established by comparison with an established "gold standard" assay method (HI, SN, CF, isolation, culture, necropsy, etc.). The minimum detectable level of reactivity, expressed as titer of antibody or nanograms of antigen, number of worms, or other appropriate measures should be established. This testing should include several known negative, weak positive, and strong positive sera or antigen preparations.

B. Specificity - Expressed as the percentage of negative results from specimens obtained from animals known to be disease free. Kits for the detection of antibody should be tested against immune sera to related agents or antigens to detect crossreactivity. The kit should also be tested against sera from animals that have received commonly used immunizing products. Kits for the detection of antigens should be tested against other related antigens to detect false positive reactions.

C. Suitability - Experimental kits should be evaluated in typical laboratory settings; at least three laboratories in different geographic locations are recommended. Adequacy of directions for use and the interpretation of results should be determined.

D. Reproducibility - For quantitative test kits with antigen or antibody fixed to a plate, or other solid phase, controls consisting of negative, weak positive, and strong positive samples should be tested in quadruplicate on at least five different solid phase units to establish the reactivity for each control. The coefficient of variation must be within acceptable limits for the planned application of the test.

VI. SERIAL RELEASE TESTING

A. Bacteria and fungi. Finished kit components and reagents are exempt from tests prescribed in 9 CFR, Parts 113.26, 113.27 and 113.28.

B. Safety Tests - Finished kit components and reagents are exempt from animal tests for safety.

C. Potency Tests - Potency tests are performed on each serial of assembled kits; such tests provide assurance that each component is functioning properly. The potency test is performed as specified in the insert supplied with the kit or as specified in the filed Outline of Production. The reactivity of the control sera included in the kit, expressed as absorbance, must be established and specified in the Outline of Production. The control sera must test within the range(s) specified.

Reference preparations which are not a part of the kit must be tested with each serial. Reference preparations should be identified in the Outline of Production by lot number and date of preparation. The minimum and maximum reactivity of the reference, e.g., titer of antibody, or nanogram of antigen, must be correlated with the sensitivity of the kit as established by prelicensing testing; and must be specified in the Outline of Production; sufficient reference preparations should be used to adequately evaluate the sensitivity and specificity of each serial. In addition, test kits approved for use in State - Federal control\eradication programs must achieve a passing score on the proficiency check-set test panel supplied by the National Veterinary Services Laboratories. Serials are eligible for release if all applicable tests are satisfactorily completed as specified in the Outline of Production.

/s/

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